similar to the size of a mammalian cell. We studied the process of filament alignment and found that it depends critically upon filament length and density. Fluorescence video microscopy and image analysis allowed the time course of filament alignment and the formation and disappearance of oriented domains to be measured. Domains of oriented filaments formed spontaneously and were separated by distinct boundaries. The pattern of the domain structures changed on the time scale of several seconds and the collision of neighboring domains led to emergence of new patterns. Our results indicate that actin filament crowding may play an important role in structuring the leading edge of migrating cells. We propose that self-alignment of actin filaments may make an important contribution to cell polarity and provide a mechanism by which the cell migration might respond to chemical cues.

12-Subg
Regulation of the Motor Function of S. cerevisiae Kinesin-5 proteins
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The conserved Kinesin-5 motor proteins play essential roles in the mitotic spindle morphogenesis. Although the Kinesin-5 functions were demonstrated in various stages of mitosis, the mechanisms that regulate their intracellular activity and motile properties have not yet been elucidated. In the present study, we examined anaphase B functions [1-3], regulation of intracellular activity and motile properties of two *S. cerevisiae* Kinesin-5 homologues, Cin8 and Kip1. We show that Cin8 and Kip1 exhibit different localization patterns to the mitotic spindle during anaphase B. We also show that Cin8 is differentially phosphorylated during anaphase at the Cdc2-kinase phosphorylation sites, located in its motor domain. To study the regulation of the motile properties of Cin8 and Kip1, we examined their microtubule binding and motility directly from cell-extracts, by the single-molecule assay. We found that Cin8 is faster compared to Kip1 and that Cin8's motile properties are dependent on a unique 99 amino-acid insert in its catalytic domain. Finally, we show that phosphorylation of Cin8 regulates its localization to the anaphase spindles and affects spindle elongation rates and morphology. Based on these findings, we propose a model for regulation of Kinesin-5 function during mitotic spindle morphogenesis.
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13-Subg
Small Loops Make a Big Difference: Insight into the Molecular Mechanism of the Multi-Tasking Kinesin-8 Motor
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Kinesins are ATP-dependent nanomotors that perform many essential microtubule based activities in the cell. Although a conserved ATPase activity and mode of microtubule binding defines them as kinesins, modifications of these core properties produce different functions within the motor superfamily.
Members of the kinesin-8 motor class have the remarkable ability to both walk towards microtubule plus-ends and depolymerise these ends on arrival, thereby regulating microtubule length.To dissect how kinesin-8s multi-task, we studied the structure and function of the kinesin-8 motor domain.We determined the first crystal structure of a kinesin-8 and used cryo-electron microscopy to calculate the structure of the microtubule-bound motor.Microtubule-bound kinesin-8 reveals a novel conformation compared to the crystal structure, including a bent conformation of the α4 relay helix and ordering of functionally important loops. In particular, loop2 is extended in kinesin-8s and forms an additional point of contact with the microtubule surface. The kinesin-8 motor domain does not depolymerise stabilised microtubules with ATP but does form tubulin rings in the presence of a non-hydrolysable ATP analogue. This shows that, by collaborating, kinesin-8 motor domain molecules can release tubulin from microtubules, and that they have a similar mechanical effect on microtubule ends as kinesin-13s that enables depolymerisation. Our data reveal aspects of the molecular mechanism of kinesin-8 motors that contribute to their unique dual motile and depolymerising functions, which are adapted to control microtubule length.

14-Subg
Direct Visualization of Walking Myosin V Molecules by High-Speed AFM
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Facilitated by the processivity of myosin V, numerous single-molecule studies have demonstrated that it moves hand over hand with a ~36 nm advance for every ATP hydrolysis. However, these results are obtained using optical microscopy and therefore the molecule itself is invisible in the observations. The motor has been visualized using electron microscopy but the obtained images are static. Thus, the detailed structural dynamic of walking myosin V and its functional mechanism are not yet fully described. Here, we directly visualized walking M5-HMM (tail-truncated myosin V) using high-speed AFM with nanometer and sub-second spatiotemporal resolution. The molecular movies provided not only corroborative visual evidence for established and speculated behaviors but also revealed unappreciated ones. First, it was cleared that the forward movement is driven not by bending but by rotation of the leading head. The leading head often showed 'foot-stomping-like brief detachment and rebinding followed by forward step. In ADP, the coiled-coil tail of the two-headed bound molecule occasionally unwound, which was followed by the leading lever-arm swing, indicating the presence of intramolecular tension responsible for the powerstroke. From these observations, it is concluded that tension for forward movement can be produced without transitioning through a weak-binding ADP-Pi-bound state. Moreover, in low concentrations of ADP, the leading head showed conformational switching between the straight and sharp bend conformations, from which the rate of ADP dissociation from the leading head was estimated to be 0.1/s. This is straightforward evidence that the ATPase cycle is essentially stalled at the leading head. Thus, dynamic visualization of functioning M5-HMM by high-speed AFM lead to a comprehensive understanding of how it operates to function.

SUBGROUP: Nanoscale Biophysics

15-Subg
Novel Environmentally-Sensitive Fluorescent Probes for Nanoscale Live Cell Imaging
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Intracellular detection of environmental polarity or viscosity at high spatiotemporal resolution helps understanding subtle cellular processes. For instance, membrane polarity and viscosity are known to determine the membrane structure and tune the function of membrane protein [1]. Biomolecular binding is always associated with changes in the physicochemical properties at the interface, thus representing a promising way to detect label-free endogeneous biomolecules by an externally-administered fluorescent biosensor [2].
In this perspective, the design and engineering of new environmentally-sensitive fluorescent probes is fundamental to new high-resolution strategies of cell imaging. Ideally, such probes should fulfil these requirements: a) optical responses (intensity, wavelength-shift, lifetime) strongly and predictably related to the environmental polarity or viscosity changes, b) brightness allowing for single-molecule detection, c) easily conjugable to biomolecules, d) suitable for nanoscopy imaging strategies.
Here I will describe recent results [3,4] and ongoing research on the development of solvatochromic chromophores having good brightness and large Stokes shifts. These molecules were pre-screened by computational methods and are endowed with a) push-pull electronic groups, ensuring for the observed solvatochromic behavior, b) functional groups, to allow for biomolecular binding.
In cultured cells, these compounds showed unchanged photophysical properties and high biocompatibility [3]. Their efficiency in reporting on biomolecular binding processes has also been tested [4]. Finally, I will show that some of these compounds can be used for single-molecule/super-resolution imaging. These probes do expand the range of fluorescence intracellular imaging applications and will be followed by more carefully designed compounds, a field where my group is currently active.
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